final concentration 20 U and mixed at 4°C overnight. The mixture was centrifuged at 3,000 rpm at 4°C for 10 minutes and recovered supernatant was used as the recombinant protein [cf. Schelp et al., Appl. Parasitol. 36, 1-10 (1995)].

The obtained recombinant protein was blotted onto nitrocellulose membrane (HybondTM-C extra, Amersham). Western blotting was performed using the monoclonal antibody produced by BC11D prepared in Example 2 as a primary antibody and peroxidase-conjugated goat anti-mouse IgG antibody (manufactured by Jackson Immunoresearch Laboratories, Inc.) capable of binding to the primary antibody as a secondary antibody. As a result, it was found that the recombinant protein reacted with the monoclonal antibody produced by BC11D prepared in Example 2 and a molecular weight of the expressed protein corresponded to the 48kDa protein derived from BC protozoa (Fig. 3).

Example 5: Analysis for Distinction Between BC and BE by ELISA Using Recombinant Antigen

ELISA was performed as reported by Takumi et al. [Jpn. J. Vet. Sci. 52(2), 241-250 (1990)]. That is, the expressed protein obtained in Example 4 was diluted with 0.05 M carbonated/bicarbonate buffer (pH 9.6) and added to 96-well plate for ELISA at 50 µl/well and incubated at 4°C

overnight to immobilize the protein. After immobilization, the plate was washed once with PBS supplemented with 0.05% Tween 20 and to the plate was added PBS supplemented with 3% skimmed milk at 100 µl/well. The plate was incubated at 37°C for 60 minutes for blocking. After blocking, the plate was washed once with PBS supplemented with 0.05% Tween 20. To the plate was added samples diluted to 1/80 with PBS supplemented with 3% skimmed milk at 50 µl/well and the plate was incubated at 37°C for 60 minutes. The samples used were serum from horses experimentally infected with either BC or BE and equine serum infected with neither of BC nor BE prepared in the Racing Horse Comprehensive Laboratory, Japan Racing Association. After completion of reaction, the plate was washed six times with PBS supplemented with 0.05% Tween 20 and to the plate was added peroxidase-conjugated anti-horse IgG antibody (manufactured by Cappel) diluted to 1/4,000 with PBS supplemented with 3% skimmed milk at 50 µl/well. The plate was incubated at 37°C for 60 minutes. After completion of reaction, the plate was washed six times with PBS supplemented with 0.05% Tween 20. To the plate was added a solution of 0.1M citric acid, 0.2M sodium phosphate, 0.003% hydrogen peroxide and 0.3 mg/ml 2,2'-azino-bis(3-ethylbenzothizolin-6-sulphonic acid)(manufactured by Sigma) at 100 µl/well. The plate was incubated at room temperature for 60 minutes and thereafter

absorbance at 415 nm was measured for each well. The results are shown in Table 1.

Table 1

ELISA Value of Equine Serum Infected with Neither of BC nor BE	ELISA Value of Equine Serum Experimentally Infected with BE	ELISA Value of Equine Serum Experimentally Infected with BC
0.039	0.018	0.319
0.021	0.032	0.541
0.003	0.045	0.805
0.014	0.033	0.700
0.029		0.721
0.020		
0.068		
0.017		

ELISA was performed with BC-negative equine serum to reveal that the ELISA had positive limitation of 0.2. As a result of ELISA using the recombinant antigen, ELISA value for equine serum infected neither with BC nor BE and for equine serum infected with BE was not more than 0.2 whereas it was 0.319 to 0.805 for equine serum infected with BC, indicating difference in specificity.